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## TABLE OF CONTENTS

No	ID	Title/Authors	Page
1	1	<b>Preparation and characterization of PLA/ALGAE nanocomposites for Packaging Industry</b> Sarah Amalina Adli, Fathilah Ali, Azlin Suhaida Azmi <i>International Islamic University Malaysia, Malaysia</i>	1
2	2	<b>Isolation and Screening of Newly Isolated Bacteria Producing Nanocellulose From Rotten Banana Skin And Sugarcane Bagasse</b> Nurul Hidayah Yusoff Sayuti, Dzun Noraini Jimat, Azlin Suhaida Azmi, Raha Ahmad Raus, Azura Amid, Parveen Jamal. <i>International Islamic University Malaysia, Malaysia</i>	5
3	3	<b>Selection of Nutrients for the Production of Myco-coagulant from Mycelial Fungus Fermentation</b> Hamidah Binti Hassan, Md. Zahangir Alam, Abdullah Al Mamun. <i>International Islamic University Malaysia, Malaysia</i>	8
4	4	<b>Immobilization of CLEA-Lipase of <i>Hevea brasiliensis</i> onto Magnetic Nanoparticles for Enhanced Biocatalytic Performance</b> Nur Amalin Ab Aziz Al Safi, Faridah Yusof <i>International Islamic University Malaysia, Malaysia</i>	11
5	5	<b>Lysozyme-Loaded Porous Hollow Core Shell Copper Zinc Oxide Nanospheres as Novel Nanoformulation for Antimicrobial Therapy</b> Farahanim Johari, Yusilawati Ahmad Nor, Maizirwan Mel, Dzun Noraini, Husna Ahmad Tajuddin <i>International Islamic University Malaysia, Malaysia</i>	16
6	6	<b>Performance Testing of 10 kW Integrated Mobile Gasifier for Sustainable Power and Biofuels Production</b> Nadly Aizat Nudri, Wan Azlina Wan A.B. Karim Ghani <i>Universiti Putra Malaysia, Malaysia.</i>	20
7	9	<b>Chitosan and Plastic Wrapping Applications to Mangosteen Fruit of Stage III in Lengthening Fruit Shelf-Life and Maintaining High Fruit Qualities</b> Zulferiyenni , Soesiladi Esti Widodo , Muhammad Kamal and Mira Lerizka <i>University of Lampung, Indonesia</i>	25
8	10	<b>Preliminary Study on Zeolite 13X as a Potential Carrier for Algal Immobilization</b> Seyed Amirebrahim Emami Moghaddam, Razif Harun, Mohd Noriznan Mokhtar, Rabitah Zakaria <i>Universiti Putra Malaysia, Malaysia</i>	28

No	ID	Title/Authors	Page
9	13	<b>Pre-treatment of Water Hyacinth for Bioethanol Production</b> Lee Xin Ling, Dayang Radiah Awang Biak, Suryani Kamarudin, Mohd Yusof Harun <i>Universiti Putra Malaysia, Malaysia</i>	31
10	15	<b>Rough Hollow Mesoporous Silica Nanoparticles as Carrier for Agarwood Oil to Treat Cancer Cells</b> Nurul Hafizah Khairudin, Yusilawati Ahmad Nor, Yumi Zuhani Has-Yun Hashim <i>International Islamic University Malaysia, Malaysia</i>	35
11	17	<b>Bioconversion of Rubber Leaves by Black Soldier Fly Larvae and Subsequent Enzymatic Hydrolysis to Produce Protein Hydrolysate</b> Mochamad Firmansyah, Nurhayati Br Tarigan, Asri Ifani Rahmawati, Muhammad Yusuf Abduh <i>Institut Teknologi Bandung, Indonesia</i>	40
12	19	<b>Screening of Long Chain Imidazolium Base Ionic Liquids for EPA and DHA Extraction from Microalgae using COSMO-RS Model</b> Shiva Rezaei Motlagh, Razif Harun, Dayang Radiah Awang Biak, Siti Aslina Hussain <i>Universiti Putra Malaysia, Malaysia</i>	43
13	20	<b>Morphologies and Mechanical Properties of Polylactic Acid / Polypropylene Carbonate (PLA/PPC) Blends by Solvent Casting Method</b> Intan Najwa Humaira Mohamed Haneef, Norhashimah Mohd Shaffiar, Yose Fachmi Buys, Malek Hamid, Sharifah Imihezri Syed Shaharuddin <i>International Islamic University Malaysia, Malaysia.</i>	47
14	22	<b>Drying Kinetics Modeling of Plectranthus Amboinicus Leaves in Tray Drier</b> Nur Amirah Asifa Raisha Zahari, Luqman Chuah Abdullah, Chong Gun Hean, Chua Bee Lin <i>University Putra Malaysia, Malaysia, Taylor's University, Malaysia</i>	51
15	24	<b>Lipid Production from Bioremediation of Palm Oil Mill Effluent</b> Ahdyat Zain Athoillah, Farah Binti Ahmad. <i>International Islamic University Malaysia, Malaysia</i>	55

No	ID	Title/Authors	Page
		<b>Techno-Economic Analysis for Integrated Stingless Bee Cultivation (<i>Tetragonula Laeviceps</i>) and Small Scale Coffee Industry in Cibodas, Indonesia</b>	59
16	25	Abdurrahman Adam, Muhammad Naufal Hakim, Lina Oktaviani, Bagoes Muhammad Inderaja, Muhammad Yusuf Abduh. <i>Institut Teknologi Bandung, Indonesia</i>	
		<b>Characterization of Biocomposites Biocellulose/Multi Walled Carbon Nanotubes for Potential Hydrogen Gas Sensor at Room Temperature</b>	62
17	26	Shahera Mohd Noor, Suryani Kamarudin, Dayang Radiah Awang Biak, Faizah Md Yasin <i>Universiti Putra Malaysia, Malaysia.</i>	
		<b>Nutrients and Chemical Oxygen Demand (COD) Removals by Microalgae-Bacteria Co-culture System in Palm Oil Mill Effluent (POME)</b>	65
18	27	Amirah Samsudin, Azlin Suhaida Azmi, Mohd Nazri Mohd Naw, Amanatuzzakiah Abdul Halim <i>International Islamic University Malaysia, Malaysia.</i>	
		<b>Media Optimization for the Production of Thermostable and Organic Solvent Tolerant Lipase by Solid State Fermentation from Palm Kernel Cake</b>	69
19	28	Hidayah Zubairi, Md Zahangir Alam, Md Noor Salleh, Hamzah Mohd Salleh <i>International Islamic University Malaysia, Malaysia</i>	
		<b>Optimization of <i>Citrus suhuiensis</i> Suspension Culture Conditions Using Response Surface Methodology (RSM)</b>	72
20	29	Nur Alia M. Fathil, Noor Illi Mohamad Puad, Azlin Suhaida Azmi, Azura Amid <i>International Islamic University Malaysia, Malaysia</i>	
		<b>Study of Eco-Cleaner Production from Pineapple Waste Fermentation Using Subcritical Water</b>	76
21	30	Natasya Fauzi, Nur Syakina Jamali, Hiroyuki Yoshida, Shamsul Izhar <i>Universiti Putra Malaysia, Malaysia</i>	
		<b>Effect of Inoculum Ratio for Anaerobic Digestion of Hydrolyzed Palm Oil Mill Effluent for Biogas Production</b>	80
22	31	Nurul Alia Fazil, Md Zahangir Alam, Mariatul Fadzillah Mansor, Azlin Suhaida Azmi. <i>International Islamic University Malaysia, Malaysia</i>	

<b>No</b>	<b>ID</b>	<b>Title/Authors</b>	<b>Page</b>
23	33	<b>Screening Fungi for Lipid Production for Consolidated Bioprocessing</b> Mohd Haffizi Hasni, Farah Binti Ahmad, Ahdyat Zain Athoillah, Mariatul Fadhillah Mansor <i>International Islamic University Malaysia, Malaysia.</i>	83
24	35	<b>Preliminary Study for Lipid Extraction from Fungi Biomass of <i>Aspergillus Oryzae</i></b> Alia Tasnim Hazmi, Farah Binti Ahmad, Ahdyat Zain Athoillah, Ahmad Tariq Jameel <i>International Islamic University Malaysia, Malaysia</i>	86
25	38	<b>Antioxidant Capacity and Characterization of Gum Arabic Crude Methanol Extract and It Is Fractions by Fourier Transform Infrared (FTIR) and Raman Spectroscopy</b> Ahmed Mohammed Elnour, Mohamed Elwathig Saeed Mirghani, Nasreldin Ahmed Kabbashia, M.D Zahngir Alam, Khalid Hamid Musa. <i>International Islamic University Malaysia, Malaysia, College of Agriculture and Veterinary Medicine Qassim university, Saudi Arabia, University of Kordofan, Sudan</i>	90
26	39	<b>Effect of Acid Hydrolysis Treated Pineapple Fiber in Plasticized Polylactic Acid Composite</b> Sharifah Nur Aqilah Binti Wan Osmani, Fathilah Binti Ali, Sarah Amalina Adli <i>International Islamic University Malaysia, Malaysia</i>	94
27	40	<b>Quantifying <i>E. coli</i> O157:H7 via Linear Sweep Voltammetry in Phosphate Buffered Saline and Tap Water</b> Nasteho Ali Ahmed, Nurul Izzati Ramli, Nurfatin Azma Abdul Jalal, Firdaus Abd Wahab, Wan Wardatul Amani Wan Salim <i>International Islamic University Malaysia, Malaysia</i>	98
28	41	<b>Nanocatalyst from Waste for Biodiesel Production</b> Nor Azyan Farrah Adila Zik, Sarina Sulaiman, Parveen Jamal. <i>International Islamic University Malaysia, Malaysia</i>	102
29	42	<b>GC-MS Analysis, Phytochemical Screening, Antimicrobial and Antioxidant Activity of Sudanese <i>Annona squamosa</i> Seeds Oil</b> Tuhami Hager, Abdelrahman Almain, Manhal Mohammed, Mohamed Elwathig Mirghani, Ahmed Mohammed Elnour. <i>International Islamic University Malaysia, Malaysia</i>	107

No	ID	Title/Authors	Page
30	43	<b>Effect of Temperature, pH and Light Intensity to Biodesalination of Seawater by <i>Synechococcus sp.</i> PCC 7002</b> Fazrizatul Shakilla Sani, Azlin Suhaida Azmi, Fathilah Ali <i>International Islamic University Malaysia, Malaysia</i> <i>University of Malaya, Malaysia</i>	109
31	44	<b>Enhancement of Biogas Production from Sewage Sludge by Biofilm Pretreatment Method</b> Ibrahim Gamiye Bouh, Md Zahangir Alam, Nassereldeen Ahmed Kabbashi <i>International Islamic University Malaysia, Malaysia</i>	113
32	46	<b>Optimization Study on Decolorization Process of Oyster Mushroom's Chitin Thin Film</b> Najiha Syahida Mohd Nizam, Wan Mohd Fazli Wan Nawawi, Wan Amalina Rosli, Ira Shahira Zailin <i>International Islamic University Malaysia, Malaysia</i>	117
33	47	<b>Optimization of Enzymatic Pretreatment Process for Enhanced Biogas Production from Palm Oil Mill Effluent</b> M. S Tajul Islam, Md Zahangir Alam, Abdullah al Mamun <i>International Islamic University Malaysia, Malaysia</i>	120
34	48	<b>Antimicrobial Properties of Composite Film Based Polyvinyl Alcohol/Starch/Nanocellulose from Sugarcane Bagasse Coated with Essential Oil</b> Maimunah Asem, Dzun Noraini Jimat <i>International Islamic University Malaysia, Malaysia</i>	124
35	49	<b>Synthesis and Characterization of Bionanocomposite Foam PVA-Based Reinforced with Sugarcane Bagasse Nanocellulose</b> Sharifah Shahira Syed Putra, Dzun Noraini Jimat <i>International Islamic University Malaysia, Malaysia</i>	127
36	50	<b>Synthesis and Process Parameter Optimization of Immobilizing CLEA-Lipase from Fish Viscera onto Magnetic Nanoparticles</b> Hanufa Khatun, Faridah Yusof, Nor Fadhillah bt Mohamed Azmin <i>International Islamic University Malaysia, Malaysia</i>	131
37	51	<b>Optimization of The Amino Functionalization of Magnetic Nanoparticles for Use in MNP-CLEA Technology</b> Hanufa Khatun, Faridah Yusof, Nor Fadhillah bt Mohamed Azmin <i>International Islamic University Malaysia, Malaysia</i>	135
38	52	<b>Municipal Wastewater Treatment by <i>Chlorella vulgaris</i></b> Nur Salwani Hasan, Azlin Suhaida Azmi, Sarina Sulaiman, Dzun Noraini Jimat <i>International Islamic University Malaysia, Malaysia</i>	139

No	ID	Title/Authors	Page
		<b>Physicochemical Characteristics of Struvite Crystals Formed in Palm Oil Mill Effluent Anaerobic Digester</b>	143
39	53	Muzzammil Ngatiman, Mohammed Saedi Jami, Mohd Rushdi Abu Bakar, Vijaya Subramaniam, Soh Kheang Loh. <i>Malaysian Palm Oil Board, Malaysia and International Islamic University Malaysia, Malaysia</i>	
		<b>Discovery of Cold-active Protease from Antarctic Region for Bio-prospecting</b>	146
40	54	Muhammad Asyraf Abd Latip, Noor Faizul Hadry Noordin, Siti Aisah Alias, Jerzy Smykla, Faridah Yusof, Mohd Azrul Naim <i>International Islamic University Malaysia, Malaysia, University of Malaya, Malaysia, Institute of Nature Conservation, Poland.</i>	
		<b>Effect of Plant Ontogeny on Yield and Chemical Constituent Essential Oil in Sweet Basil (<i>Ocimum Basilicum</i> L.) Grown In Sudan</b>	149
41	55	Yasmin A. A. Aburigal, Elfadl Y. Elmogtaba, Mirghani, M. E. S, Awatif A. M. Siribel, Nada B. Hamza, Ismail H. Hussein <i>University of Gezira, Sudan, International Islamic University Malaysia, Malaysia, Medicinal and Aromatic Plants Research Institute</i>	
		<b>Transforming Fish Gelatin Hydrolysates into Nanoparticles as Drug Carrier</b>	152
42	57	Deni Subara, Irwandi Jaswir <i>International Islamic University Malaysia, Malaysia</i>	
		<b>The Use of Effective Microorganisms (EM) for Pretreatment of Wet Market Wastewater</b>	156
43	58	Munira Shahbuddin, Raha Ahmad Raus, Nur Amalina Ramli, Nahla Adel, Saedi Jami, Zaki Zainuddin <i>International Islamic University Malaysia, Malaysia</i>	
		<b>Issues and Challenges in the Development of Three-Dimensional Model for Thyroid Cancer</b>	161
44	59	Munira Shahbuddin <i>International Islamic University Malaysia, Kuala Lumpur, Malaysia</i>	
		<b>Screening of Boron Adsorption Potentials of Various Date Seed Preparation Methods</b>	165
45	61	Moussa Mohamed Ahmed, Mohammed Saedi Jami, Md Noor Bin Salleh, Mohamed Elwathig Saeed Mirghani <i>International Islamic University Malaysia, Malaysia</i>	



No	ID	Title/Authors	Page
46	62	<b>Achievements and Perspectives on Anaerobic Co-Digestion of Food Waste and Sewage Sludge for Biogas Production</b> Ainoor Mariana Mohd Ali, Mariatul Fadzillah Mansor, Md. Zahangir Alam. <i>International Islamic University Malaysia, Malaysia</i>	168
47	63	<b>Assessment of <i>Dicranopteris linearis</i>, <i>Nephrolepis bifurcata</i>, <i>Stenochlaena palustris</i> and <i>Acrostichum aureum</i> Heavy Metal Sequestration Rate Stability at Highly Weathered Soil Area</b> Nur Hanie Mohd Latiff, Rashidi Othman, Zainul Mukrim Baharuddin <i>International Islamic University Malaysia, Malaysia</i>	171
48	64	<b>Factors Affecting the Hydrolysis of Empty Fruit Bunches in Ionic Liquid-Compatible Cellulase System</b> Amal A.M. Elgharbawy, Md. Zahangir Alam <i>International Islamic University Malaysia, Malaysia</i>	174
49	65	<b>Development of Solid State Fermentation for Protease Enzyme Production From The Indah Water Konsortium Sludge Cake</b> Tamzeed Mahbubul Karim, Md Zahangir Alam <i>International Islamic University Malaysia, Malaysia</i>	177
50	66	<b>Optimisation of Process Conditions on The Ultrasonic Extraction of Phenolics Scavenger from Malaysian Curcuma Caesia Rhizome</b> Parveen Jamal, Siti Fathimah Putery Jemain, Irwandi Jaswir, Azura Amid, Raha Ahmad Raus <i>International Islamic University Malaysia, Malaysia</i>	180
51	67	<b>Optimization of Media Compositions of <i>Lactobacillus plantarum</i> For DPPH Scavenging Ability</b> Parveen Jamal, Siti Fathimah Putery Jemain, Irwandi Jaswir, Raha Ahmad Raus, Azura Amid <i>International Islamic University Malaysia, Malaysia</i>	185
52	68	<b>Effects of Aminoethoxyvinylglycine and Postharvest Treatment Package of Plastic Wrapping, Fungicide Prochloraz, and Low Temperature on The Fruit Shelf-Life And Qualities of 'Callina' Papaya</b> Soesiladi Esti Widodo, Muhammad Kamal, Suskandini R. Dirmawati, Zulferiyenni, Rachmansyah A. Wardhana, Luthfah Q. Aini <i>University of Lampung, Indonesia</i>	188

No	ID	Title/Authors	Page
53	70	<b>Comparison of Monoculture and Co-Culture of Microalgae for Enhanced Lutein Production</b> Raha Ahmad Raus, Nor Jannah Sallehudin, Mokhzanni Mustapa <i>International Islamic University Malaysia, Malaysia, Ministry of Health Malaysia, Malaysia</i>	191
		<b>Ionic Liquid Microwave-Assisted Extraction of <i>Annona Muricata</i> Fruit</b>	195
54	71	Djabir Daddiouaissa, Azura Amid, Nassereldeen Ahmed Kabbashi, Mohamad Adika Khairy Mohd Shaifudin Epandy <i>International Islamic University Malaysia, Malaysia</i>	
		<b>Assessment of <i>Synechococcus</i> sp. and <i>Pseudanabaena</i> sp. Cell Culture Model System for Biogenesis and Heavy Metal Sequestration Mechanism</b>	200
55	72	Rashidi Othman, Ruhul 'Izzati Shahrudin, Wan Syibrah Hanisah Wan Sulaiman, Zainul Mukmin Baharuddin <i>International Islamic University Malaysia, Malaysia</i>	
		<b>Effect of Light and PH on <i>Pandorina Morum</i> And <i>Chlorella vulgaris</i> Cell Culture Growth and Heavy Metals Sequestration Rate</b>	204
56	73	Rashidi Othman, Ruhul 'Izzati Shahrudin, Wan Syibrah Hanisah Wan Sulaiman, Zainul Mukrim Baharuddin <i>International Islamic University Malaysia, Malaysia</i>	
		<b>Newly Isolated <i>Clostridium</i> Species for Biobutanol Production: A Review</b>	207
57	74	Nur Suhaili Aris, Hafiza Shukor, Saleha Shamsudin <i>University Malaysia Perlis, Malaysia</i>	
		<b>Transesterification Process of <i>Moringa Oleifera</i> Oil to Biodiesel</b>	211
58	76	Nassereldeen Kabbashi, Yara Hunud, Mohamed Elwathig Mirghani, Mohammed, E, Saeed, Moneef M. Jazzar <i>International Islamic University Malaysia, Malaysia Arab Open University, Kuwait Branch</i>	
		<b>Optimization of Process Parameters for Boron Adsorption From Sea Water Using <i>Moringa Oleifera</i> Seeds</b>	215
59	86	Mohammed Saedi Jami, Nur Syahirah Zakaria, Moussa Mohamed Ahmed, Mohammed Ngabura, Mani Malam Ahmad <i>International Islamic University Malaysia, Malaysia, Universiti Putra Malaysia (UPM), Malaysia, Universiti Malaysia Pahang (UMP), Malaysia</i>	

No	ID	Title/Authors	Page
60	88	<b>Influence of Aeration on Nutrients Removal by <i>Chlorella sp.</i> From Wastewater</b> Nur Zarifah Mohd Isa, Maan Fahmi Rashid Al-Khatib, Farah Binti Ahmad, Raha Ahmad Raus, Fadi I.A. Alqedra <i>International Islamic University Malaysia, Malaysia</i>	218
61	89	<b>Optimization of the Production of Fish Gelatin Nanoparticles as A Carrier for A Sunflower Derived Biopeptide</b> Iqrah Akbar, Irwandi Jaswir, Parveen Jamal. <i>International Islamic University Malaysia, Malaysia</i>	221
62	90	<b>Minimization of Excess Sludge Production for Sand Mining Wastewater Treatment</b> Nurain Nazira Ramani, Husna Tajuddin. <i>International Islamic University Malaysia, Malaysia</i>	225
63	91	<b>Wastewater Treatment of Sg. Pusu Using Novel Coagulant</b> Rahayu Sulaiman, Husna Ahmad Tajuddin, Yusilawati Ahmad Nor <i>International Islamic University Malaysia, Malaysia</i>	229
64	92	<b>Activation and Optimization of Renewable Durian (<i>Durio Zabethenus Murray</i>) Shell for Zinc Biosorption from Aqueous Medium</b> Mohammed Ngabura, Siti Aslina Hussain, Mohammed Saedi Jami <i>Universiti Putra Malaysia, Malaysia</i> <i>International Islamic University Malaysia, Malaysia</i>	233
65	93	<b>Analogues of 2-Deoxyglucose as Inhibitor Candidates for Hexokinase II Identified via Virtual Screening Analyses</b> Suriyea Tanbin, Nurhainis Ogu Salim, Fazia Adyani Ahmad Fuad <i>International Islamic University Malaysia, Malaysia</i>	237
66	94	<b>Effect of Carbon-Based Nanomaterial on Production of Exopolysaccharides and Growth of Microalgae: A Review</b> Fakhriah Fakhirruddin, Habibah Farhana Abdul Guthoos, Azura Amid, Wan Wardatul Amani Wan Salim, Azlin Suhaida Azmi <i>International Islamic University Malaysia, Malaysia</i>	241
67	95	<b>Mechanical Properties, Morphology, and Hydrolytic Degradation Behavior of Polylactic Acid/Thermoplastic Polyurethane Blends</b> Yose Fachmi Buys, Mimi Syakina Ahmad, Hazleen Anuar <i>International Islamic University Malaysia, Malaysia</i>	245
68	96	<b>Metabolite Profiling of Leaves and Branch of <i>Aquilaria sp.</i></b> Nor Fadhilla Mohamed Azmin, Hamnah Sheerin, Yumi Zuhanis Has-Yun Hashim, Nur Fatimah Nabilah Rosely <i>International Islamic University Malaysia, Malaysia</i>	249

No	ID	Title/Authors	Page
69	98	<b>Medicinal Properties Screening of <i>Mallotus Paniculatus</i> Extract</b> Nur Aliah Bahaman, Raha Ahmad Raus, Yusilawati Ahmad Nor <i>International Islamic University Malaysia, Malaysia</i>	254
70	99	<b>Preliminary Study on Designing and Fabrication of Synthesis Gas Analyser in Biomass Gasification Performance</b> Muhamad Farhan Mohd Pu'ad, Khairul Azami Sidek, Maizirwan Mel <i>International Islamic University Malaysia, Malaysia</i>	258
71	100	<b>Transformation of Carbon Dioxide into Methanol</b> Nurjehan Ezzatul Ahmad, Fatin Nabilah Murad, Nur Amalina Ramli, Zulaika Muhamad Sade, Ima Shahanaz Ahmad Zailani, Mohd Sharul Amri Rosmadi, Hamzah Mohd Salleh, Maizirwan Mel <i>International Islamic University Malaysia, Malaysia</i>	261
72	101	<b>Immobilization of Lipase in Functionalised Potassium Carrageenan Beads and Optimization of the Enzyme Activity</b> Farah Nabila Fauzli, Ahmad Tariq Jameel <i>International Islamic University Malaysia, Malaysia</i>	264

## Optimization Study of Decolorization Process of Oyster Mushroom's Chitin Thin Film

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### Abstract

This study focuses on decolorizing the thin film using sodium hypochlorite (NaClO) to produce highly transparent chitin nanocomposite from chitin nanofiber. Preparation of the thin film from chitin extract of oyster mushroom (*Pleurotus ostreatus*) demonstrated in this study. Optimization of decolorization of chitin thin film in sodium hypochlorite (NaClO) was done by one-factor-at-time (OFAT) and statistical approach using Response Surface Methodology (RSM) in Design expert Software. This is to determine the optimal process conditions of decolorization based on three parameters which are concentration of NaClO, duration and temperature of the process. The optimization was conducted on the basis of response mechanical properties (tensile strength) of chitin thin film. The OFAT method indicated that the optimum condition for decolorization of thin film was at concentration of 6%, duration of 4 hours and temperature of 30°C with the tensile stress value of 52.57 MPa.

**Keywords:** chitin nanofiber, *pleurotus ostreatus*, decolorization, optimization, Response Surface Methodology, tensile stress

### 1. INTRODUCTION

Chitin is a tough, semi-transparent substance which acts as the building material that gives strength to the exoskeletons of crustaceans, insects and the cell walls of fungi. It can be found abundantly in nature apart from cellulose. Due to their huge sources in nature, research field on chitin based materials has been widened and expanded. Chitin nanofiber has properties of biodegradable, biocompatible, relatively inert and high mechanical properties [1]. Due to its nano- sized structure, higher surface area, outstanding mechanical properties such as high Young's modulus and fracture strength, the chitin nanofiber have strong potential in developing the nanofiber and chitin technology [2]. Chitin is a linear polymer consisting of  $\beta$ -(1-4) *N*-acetyl-D-glucosamine structure. Chitin nanofiber network in the cell walls of mushrooms is embedded in the matrices mainly of  $\beta$ -1, 3 glucans. Chitin can be disintegrated into nanofibers by several mechanical treatments such as grinder [1], high speed blender [4] and high pressure homogenizer [5]. The width of the nanofibers in the mushroom are ranging from 20-28 nm depends on its type [2]. Since this biopolymer can be found abundantly in nature and has biocompatible properties, it contributes in major application of biotechnology including biomedicine, pharmaceutical and agro-biosciences as well as food industry.

### 2. MATERIALS AND METHODS

#### A. Materials

Oyster mushrooms (*Pleurotus ostreatus*) were used as the raw material in this study. The oyster mushrooms were purchased at NSK Selayang in Selangor. The collection of the mushroom was made on 4th February 2018.

#### B. Chitin extraction from *Pleurotus ostreatus*

The oyster mushrooms were blended using high speed blender (Vita Mixer Innofood, 19000 rpm, power consumption 1500W) for 5 minutes after rinsed with distilled water. Chitin extraction process began with the hot water extraction. The blended mushroom slurry was heated up to 85°C for 1 hour during hot water extraction. Water-soluble compounds were removed in this process. Deproteinization step was carried out for protein removal by adding 1 M NaOH to the mushroom slurry and been heated up to 65°C for 3 hours. The suspension then was filtered and neutralized using distilled water until pH becomes neutral. The stock chitin extract placed in a container and stored in 4 °C.

#### C. Preparation of Chitin Thin Film

Preparation of chitin thin film started with filtration of chitin solution for 1.5 hours using filter paper (Sartorius 1288) by setting up vacuum pump (GAST, DOA-P604-BN). After filtration, the chitin wet cake was placed in between cotton cloth to reduce moisture content. Two metal plates are to be placed on the top and the bottom of cotton cloth to make sure the chitin

cake does not move. The chitin wet cake then left completely dried under the 5 kg weight at 120°C for 3 hours in the oven. After the drying process, 80 gsm of chitin thin film with the diameter of 110 mm and 0.76g are prepared.

#### *D. Decolorization of chitin thin film*

The chitin thin films with 60 mm length and 10 mm width were bleached in NaClO solution under three different process parameters which are concentration, duration and temperature of the process. In first OFAT, the concentration is varied 4%, 6%, 8%, 10% but fixed the duration at 4 hours and temperature of 28°C. For the second OFAT, the duration is varied for 2 h, 4 h, 6 h and 8 h but fixed concentration at 6% and temperature 28 °C. In third OFAT, the temperature is varied at 25°C, 30°C, 35°C, 40°C but the concentration and the duration is fixed at 6% and 4 hours respectively.

#### *E. Tensile test of chitin thin film*

The specimens were cut into rectangular pieces with the dimension of 60 mm long and 10 mm wide. Both ends of each specimen were tabbed with mounting board (1 mm thick) using epoxy adhesive glue (Araldite® Standard) to avoid sample from damage at grip point and slippage during the test. The samples were kept inside the silica chamber to ensure the constant humidity at room temperature (28% RH, 28°C). Universal tester (SHIMADZU, AGS-X, 5 kN load cell) with sample grip pressure of 4 bar was used for the tensile test according to ASTM D882-12. The specimen gage length and test speed is set to 30 mm and 1 mm/min, respectively. Five replicates of each thin film were tested for each run.

### **3. RESULTS AND DISCUSSION**

#### *A. Chitin Extraction from Oyster Mushroom*

The dry weight of the mushroom was determined to be 7% and this value was used throughout this project since the mushroom are originated from the same species and purchased at the same place. After blending, light brown mushroom slurry was obtained and then was transferred into beaker for hot water extraction. The mushroom slurry containing fiber became more viscous as a result of water removal. The extract slurry changed from pale brown to dark brown after addition of NaOH during the deproteinization process. As the protein was removed in this process, the slurry became less viscous. During neutralization process the dark brown colour started to become light brown colour again as the pH becomes neutral (6-7) after the addition of distilled water. Chitin content from 1 kg of waste oyster mushroom was found to be in the range of 3-5% (w/w).

#### *B. Preparation of Chitin Thin Film*

The process started by filtering 0.7% chitin solution for 1.5 hours. Fabrication of thin film completed by drying in the oven at 120°C for 3 hours under 5 kg weight. The thin film left dried to remove the water completely. The weight press is important to ensure the final chitin thin film produce no wrinkles. 1 kg of mushroom produced about 20 pieces of 80 g/m<sup>2</sup> thin film approximately with a mass of 0.76 g and diameter of 110 mm.

#### *C. Decolorization of chitin thin film*

Chitin thin film shows translucent properties upon decolorize in sodium hypochlorite solution (refer Fig.1). The transparency of this natural polymer also does not lose since fibers which have a diameter less than one-tenth of the visible light are not affected by light scattering [3].



Figure 1: Translucent chitin thin film: treated with 6% NaClO for 4 h at 28°C

#### *D. Mechanical properties of thin film*

Table 1-3 shows the response of tensile properties for the three OFAT. The first OFAT was to determine the influence of different NaClO concentration at room temperature (28 °C) for 4 hours. From Table 2, decolorization of thin film in 6% NaClO gave the highest tensile strength (51.33 MPa). As the NaClO concentration increased, the strength of chitin decreased. This is possibly due to chitin chain degradation. However, no significant difference for tensile modulus among the concentrations was observed except only for 10% concentration which had very low tensile modulus. The second OFAT show the influence of process duration at fixed NaClO concentration (6%) and temperature (28 °C). From Table 3, it was observed that 4 hours of duration yielded highest tensile stress (51.71 MPa). Longer duration of decolorization caused the chitin thin films lose its mechanical strength. The significant difference was in tensile modulus comparison. The influence of temperature during decolorization using 6% NaClO for 4 hours for the final OFAT was summarized in Table 4. Increase of temperature during decolorization resulting in an increase of both stress and strain until maximum at 30 °C reached (54.66 MPa and 8.32%). The mechanical strength started to decline at temperature of 35 °C. At this temperature, the thin film as in a fragile condition and easily teared up. Based on the result of these three OFAT, the optimum condition for decolorization process in sodium hypochlorite solution

Table 1: Mechanical properties of chitin thin film in different concentration of NaClO for 4 h at 28° C

Conc. (%)	Tensile Stress (MPa)	Tensile strain[%]	Tensile Modulus[MPa]	Tensile Toughness (MJ/m <sup>3</sup> )
4	27.09± 0.36	1.38±0.02	21.20±2.28	16.20±0.53
6	51.33± 2.51	6.4 ±0.19	22.50±3.42	66.99±2.42
8	41.57±2.46	4.55±0.004	15.34±3.66	42.21±1.66
10	21.24±0.88	6.81 ±0.45	5.70±0.31	52.09±1.07

Table 2: Mechanical properties of chitin thin film at different duration in 6% concentration of NaClO at 28°

Duration (h)	Tensile Stress (MPa)	Tensile strain[%]	Tensile Modulus[MPa]	Tensile Toughness (MJ/m <sup>3</sup> )
2	30.96± 4.10	4.19±0.79	16.58±1.61	61.48±1.44
4	51.71± 2.22	2.83±0.46	29.84±2.89	80.63±3.65
6	23.17±0.48	4.91±1.79	7.89±2.30	51.90±0.45
8	34.61±11.60	4.58 ±0.38	15.22±5.78	122.07±1.43

Table 3: Mechanical properties of chitin thin film at different temperature of 6% NaClO concentration at 4 hour

Temp. (°C)	Tensile Stress (MPa)	Tensile strain[%]	Tensile Modulus[MPa]	Tensile Toughness (MJ/m <sup>3</sup> )
25	45.61± 6.63	3.88±0.13	23.07±2.62	117.09±6.93
30	54.66± 0.72	4.73 ±0.89	25.81±2.05	125.15±3.44
35	24.57±0.70	3.64±1.02	11.14±3.42	64.61±1.76
40	20.81±10.44	1.69±0.42	14.21±5.63	18.94±1

(NaClO) was at 6% concentration, 4 hours duration of process and 30 °C temperature of process. These results become the basis for the optimization process later on.

#### 4. CONCLUSION

Chitin nanofibers were extracted from the oyster mushroom, *Pleurotus ostreatus* and thin films were prepared from the extracted chitin. This study aimed to determine the effect of decolorization of the chitin thin film in sodium hypochlorite (NaClO) towards its mechanical properties. The chosen parameters during decolorization were the concentration of NaClO, duration and temperature of the process with the response of mechanical properties which is tensile strength. The optimum decolorization condition by OFAT method was found to be 6% NaClO concentration, 4 hours at 30 °C with the value of tensile stress 52.57 MPa. The range of chitin content in 1 kg of mushroom was 3.0-5.0 % (w/w). Fabricated chitin thin film possesses translucent properties upon been decolorized due to nano effect of fiber. Chitin that has been undergoing decolorization process has high mechanical properties and it can be applied in making strong and transparent nanocomposite.

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